Glucocorticoid-mediated responses of plasma ACTH and anterior pituitary pro-opiomelanocortin, growth hormone and prolactin mRNAs during adjuvant-induced arthritis in the rat

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ABSTRACT

Adjuvant arthritis (AA) in the rat leads to chronic stimulation of the hypothalamic-pituitary-adrenal (HPA) axis and the loss of its diurnal rhythmicity. We have investigated the effects of adrenalectomy (ADX) and different levels of corticosterone replacement upon plasma ACTH levels and anterior pituitary pro-opiomelanocortin (POMC), GH and prolactin mRNAs during the development of AA. In control ADX animals, we observed the negative feedback effects of exogenous corticosterone on plasma ACTH and anterior pituitary POMC mRNA. In the ADX animal with AA, however, the increased POMC mRNA which was observed was not reduced by exogenous corticosterone on day 7 of AA, although the negative feedback effect of corticosterone on plasma ACTH was intact. On day 14, however, even high dose corticosterone replacement failed to have a significant feedback effect on the raised levels of plasma ACTH.

In control ADX animals, corticosterone replacement resulted in increased anterior pituitary GH mRNA and reduced prolactin mRNA. In contrast, in ADX animals with AA, GH mRNA was reduced and there was a further decrease in prolactin mRNA. In these animals, corticosterone replacement did not affect GH or prolactin mRNA expression.

These data demonstrate a disruption of the normal mechanisms underlying feedback inhibition of the HPA axis by glucocorticoids during AA. Similarly, the glucocorticoid-dependent regulation of GH and prolactin mRNA expression is altered in AA.

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INTRODUCTION


We have previously shown that the development of AA is accompanied by chronic activation of the hypothalamic-pituitary-adrenal (HPA) axis, with increased morning adrenocorticotropic hormone (ACTH) and corticosterone levels, together with abolition of the normal diurnal rhythms of ACTH and corticosterone (Sarlis et al. 1992). Anterior pituitary pro-opiomelanocortin (POMC) mRNA levels are also increased during the development of AA (Stephanou et al. 1992). Thus, in this model of chronic inflammatory stress, the drive to ACTH secretion is increased, despite the chronic increase

in corticosterone secretion. Recent studies have, however, shown that the hypothalamic expression of corticotropin-releasing hormone (CRH) mRNA and CRH 1-41 peptide levels in the hypophyseal portal venous blood are not increased in animals with AA (Harbuz et al. 1992a), suggesting that the primary drive to the HPA axis is not hypothalamic CRH (Harbuz et al. 1992b). Plasma levels of both growth hormone (GH) and prolactin are also affected by the development of AA. Prolactin secretion increases early on and then drops sharply prior to the clinical manifestations of the disease, while GH levels have been shown to be reduced (Neidhart & Larson, 1990).

The inhibitory effects of pharmacological doses of glucocorticoids on normal growth patterns have been documented both in experimental animals (Evans et al. 1943) and in humans (Blodgett et al. 1956). Patients with hypercortisolaemia due to Cushing’s disease or long-term glucocorticoid therapy have reduced GH secretion (Krieger & Glick, 1972). In contrast, recent reports suggest that glucocorticoids may have permissive effects on stimulated GH release, depending on the duration of pituitary exposure to the steroids (Casanueva et al. 1988, 1990). In vitro studies have demonstrated a stimulatory effect of dexamethasone on GH secretion and GH mRNA accumulation in somatotrophs (Levy & Lightman, 1988). Both CRH and ACTH have also been reported to increase GH secretion during the day, although not at night, suggesting that the level of activity of the HPA axis may play a role in determining GH secretion patterns (Wiederman et al. 1991). The prolactin response, however, is quite different, and glucocorticoids have been shown to have an inhibitory effect on both prolactin secretion (Kiern et al. 1990) and mRNA expression (Somasekhar & Gorski, 1988).

In this paper, we have studied the effects of corticosterone pellet replacement in adrenalectomized (ADX) animals on plasma ACTH and anterior pituitary POMC, GH and prolactin mRNAs, to analyse further the changes in negative feedback regulation of the HPA axis during the development of AA, and the associated changes in the regulation of pituitary GH and prolactin.

**MATERIALS AND METHODS**

**Animals**

Male Sprague-Dawley rats (~60 days of age; 230–270 g), kept in a cycle of 12 h light:12 h darkness (lights on at 07.00 h), were used for all experiments. Laboratory chow and tap water were available *ad libitum*, and animals were housed six to a cage.

**Treatments**

Adjuvant arthritis was induced in the animals by an intradermal injection of 0.1 ml of a suspension of ground heat-killed *Mycobacterium butyricum* (10 mg/ml; Difco, Detroit, MI, U.S.A.) in paraffin oil (Fluka Chemie AG, Buchs, Switzerland) into the tail base. Control animals were injected with vehicle alone.

Adrenalectomy or sham-ADX was performed via the dorsal approach under fentanyl citrate (0.315 mg/ml):fluanisone (10 mg/ml):diazepam (5 mg/ml) anaesthesia (1:1:2, by vol.; 0.8 ml/kg body weight). ADX rats were treated with corticosterone (Sigma, Poole, Dorset, U.K.) pellets that weighed approximately 150 mg and were composed of a fused mixture of corticosterone in cholesterol (20%, 40% or 80% corticosterone, w/w), as previously described (Meyer et al. 1979; Akana et al. 1985). Sham-ADX rats and ADX rats not receiving corticosterone were implanted with pellets of the same weight (150 mg) containing cholesterol only. Pellets were implanted s.c., slightly rostral to the skin incision made for ADX. After removal of the adrenals, rats received 0.9% (0.15 M) NaCl as drinking water. Adjuvant or vehicle injection and/or corticosterone pellet implantation were performed at the time of surgery.

**Experimental design**

Each experimental group consisted of six animals. The groups used are shown in Table 1.

All animals were decapitated within 3 h of lights-on in the morning, trunk blood was collected and plasma was stored at −20°C for subsequent hormone measurements. At the time of decapitation, the anterior pituitary glands were rapidly removed and frozen on dry ice for subsequent Northern analysis. Hind paws were amputated below the knee and the position of the lateral malleolus was marked with waterproof ink for subsequent plethysmography.

**Radioimmunoassay (RIA)**

The method for extracting ACTH from rat plasma by Sep-pak C18 cartridges, its subsequent measurement by RIA, and the corticosterone assay have been fully described previously (Mihaléy et al. 1981; Jessop et al. 1989). The intra- and interassay coefficients of variance (C.V.) for ACTH measurements were 9% and 13.5% respectively. The intra- and interassay C.V. values for corticosterone measurements were 4.5% and 9.8% respectively.

**Northern blot analysis**

Total RNA was extracted from the anterior pituitary in the presence of guanidinium thiocyanate–phenol–
The experimental groups used in this study: ADX = adrenalectomy; ADX/AA = adrenalectomy plus adjuvant injection; C20, C40, C80 = ADX animals given 20, 40 and 80% corticosterone replacement pellets respectively; AA20, AA40, AA80 = ADX/AA animals given 20, 40 and 80% corticosterone replacement pellets respectively. + and − designate the presence or absence of a particular treatment; Chol designates the administration of pellets containing cholesterol only.

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(Chloroform (Chomczynski & Sacchi, 1987), denatured with glyoxal (6 M) and dimethyl sulfoxide (50%), and electrophoresed through a 1.2% agarose gel in 12 mM Tris, 6 mM sodium acetate and 0.3 mM EDTA buffer (pH 7.0). After electrophoresis, RNA was transferred to filters (GenScreen Plus; Du Pont, Stevenage, Herts, U.K.) and fixed by drying at room temperature.

Hybridization was carried out using anti-sense oligonucleotides directed to the exonic regions coding for the rat POMC, prolactin, GH and α-tubulin genes (Department of Biophysics, King’s College, London, U.K.). The α-tubulin probe was used to assess the amount of RNA loaded per lane. The oligoprobes were labelled with [α-32P]dCTP (Amersham International plc, Amersham, Bucks, U.K.) using the nucleotide transferase enzyme (Gibco BRL, Paisley, Strathclyde, U.K.). Hybridization buffer was composed of 1 M NaCl, 1% sodium dodecyl sulphate (SDS) and denatured mechanically-sheared salmon sperm DNA (100 µg/ml). Hybridization was performed for 24 h at 60°C and the filters were then washed in 2 × SSC/1% SDS for 30 min at 60°C, followed by a wash in 0.1 × SSC for 30 min at room temperature (1 × SSC is NaCl, 150 mmol/l; tri-sodium citrate, 15 mmol/l; pH 7). Filters were then exposed to a Kodak XAR-5 X-ray film (Sigma).

 Autoradiograms were scanned with an LKB scanning densitometer (Rockville, MD, U.S.A.). The amounts of POMC, prolactin and GH mRNA were corrected for differences in recovery based on the amounts of α-tubulin mRNA, and were expressed as percentages of controls (the optical densities of controls were normalized to the α-tubulin mRNA spotted per lane). In the semiquantitative assessment of Northern blots, data are presented as means ± s.d. of results from three separate experiments.

Clinical assessment

The hind paws of animals were submerged in the plethysmometer bath (model ΔV-3; Ugo-Basile, Milan, Italy) to the level of the lateral malleolus and the mean of three recordings per paw was noted as the paw volume. The accuracy of the measurements was ±0.01 ml.

Result presentation, calculation and statistics

Results from RIAs are means ± s.e.m. and data from Northern blot analysis are means ± s.d. Statistical analysis of the ACTH data obtained from ADX/AA animals, as well as the ACTH, corticosterone and anterior pituitary POMC mRNA data obtained from ADX animals that received corticosterone pellet replacement was performed using Dunnett's range test following one-way analysis of variance (ANOVA) on comparisons of each time-point following adjuvant injection with respective controls. Newman–Keuls post-hoc test was used to assess variability among the previous groups. Paw volume data were analysed using Duncan's multiple range test following one-way ANOVA. For all comparisons, a P value of <0.05 was considered to be significant (Zar, 1984).

RESULTS

ACTH in ADX/AA animals

In ADX/AA animals on days 7 and 14 after adjuvant injection, circulating ACTH levels were not significantly different from those observed in ADX animals (Fig. 1).

ACTH and corticosterone in animals treated with corticosterone pellets

On day 7, in animals given pellets containing 20, 40 and 80% corticosterone (C20, C40 and C80), plasma corticosterone had reached levels in proportion to the concentration of the s.c. implanted pellet. By comparison with data from our previous studies of
the diurnal rhythm in control animals (Sarlis et al. 1992), we found that the pellet containing 20% corticosterone gave plasma corticosterone levels approaching those normally occurring in the circadian nadir (morning), the pellet containing 40% corticosterone resulted in levels similar to those at the circadian peak (evening), and the pellet containing 80% corticosterone resulted in levels similar to those encountered in mild stress. Plasma corticosterone levels in these animals corresponded to the well-known negative feedback effects on plasma ACTH, as progressively higher levels of corticosterone resulted in a dose-dependent reduction of ACTH levels (Fig. 2).

In the AA animals given pellets containing 20, 40 and 80% corticosterone (AA20, AA40 and AA80), the pellets reduced plasma ACTH on day 7 by amounts similar to those in the C20, C40 and C80 animals respectively. However, in these animals, this negative feedback effect was no longer apparent by day 14. For any given corticosterone pellet strength (i.e. 20, 40 or 80%), there was a gradual increase in plasma ACTH levels during the development of AA (Fig. 3).

**Anterior pituitary POMC mRNA in ADX/AA animals**

In the C20 and C40 animals on day 7, POMC mRNA was reduced by corticosterone in comparison with animals receiving ADX alone. Furthermore, the C80 animals showed a greater reduction in POMC mRNA by corticosterone in comparison with C20 and C40 animals. In the AA20, AA40 and

*Journal of Molecular Endocrinology* (1992) 9, 273–281
Anterior pituitary GH mRNA in ADX/AA animals

In the C20, C40 and C80 animals, the accumulation of pituitary GH mRNA by day 7 increased slightly, in proportion to the increasing corticosterone pellet dose. In the AA20, AA40 and AA80 animals, pituitary GH mRNA content was reduced in comparison with the C20, C40 and C80 animals and no effect of corticosterone replacement was discernible (Fig. 4). The semiquantitative analysis of these results is shown in Fig. 5.

Anterior pituitary prolactin mRNA in ADX/AA animals

In the C20, C40 and C80 animals, increasing corticosterone pellet dose resulted in slightly reduced prolactin mRNA accumulation by day 7. In the AA20, AA40 and AA80 animals, a further decrease in prolactin mRNA accumulation was also observed during the development of AA. These effects were unaffected by the dose of the corticosterone pellets (Fig. 4). The semiquantitative analysis of these results is shown in Fig. 5.

Effects of corticosterone replacement on paw volume

The paw volume of ADX/AA animals on day 14 after injection was significantly greater than the paw volume of the control animals.
volume observed in AA animals at the same time-point. Both volumes were significantly raised in comparison with control animals (Fig. 6).

In the AA20, AA40 and AA80 animals on day 14 after injection, the volume of the inflamed paws was reduced by the administration of corticosterone pellets in a dose-dependent manner, the paw volume being inversely correlated with plasma corticosterone levels (Fig. 7).

**DISCUSSION**

We have previously shown that in intact AA animals, pituitary POMC mRNA and circulating ACTH and corticosterone levels are all increased, with loss of the normal diurnal rhythm of plasma ACTH and corticosterone (Sarlis et al. 1992; Stephanou et al. 1992). Both acute and chronic non-inflammatory stresses result in an increase in the secretion of ACTH from the pituitary (Henkin & Knigge, 1963; Mikulaj & Mitro, 1972; Cook et al. 1973; Reigle, 1973; Ruhmann-Wennhold & Nelson, 1977) and consequent secretion of corticosterone from the adrenals, which in turn has feedback effects in reducing ACTH secretion from the pituitary (Bohus, 1969; Dallman & Yates, 1969; Keller-Wood & Dallman, 1984). The combination of elevated anterior pituitary POMC mRNA and plasma ACTH and corticosterone levels during the development of AA demonstrates an underlying dysfunction of corticosterone-dependent negative feedback.

The disruption of normal corticosterone-mediated negative feedback first became evident on day 7 at the level of pituitary POMC mRNA expression and by day 14 at the level of plasma ACTH. Furthermore, both plasma ACTH and pituitary POMC mRNA levels increased with time during the development of AA, irrespective of the dose of corticosterone replacement. This pattern of change closely follows the situation observed in intact AA animals (Sarlis et al. 1992).

In AA animals, in addition to the stimulation of the HPA axis, there is also activation of the immune system (Connoly et al. 1988; Whitehouse, 1988; Leisten et al. 1990). We have recently shown that interleukin (IL)-1β mRNA expression in the spleen is increased during the development of AA, and
that this is further enhanced in ADX/AA animals (Stephanou et al. 1992). Corticosterone therefore must exert negative effects on the expression of the IL-1β gene in splenocytes. IL-1β, tumour necrosis factor-α and IL-6 plasma levels are also increased in AA animals (Shinmei et al. 1989), and these cytokines have been shown to act at both the hypothalamic and pituitary level to activate the HPA axis (Brown et al. 1987; Evans, 1989; Navarra et al. 1990; Snick, 1990; Suda et al. 1990). IL-1β and IL-6 administered intravenously can also cause increased release of ACTH and corticosterone into the peripheral circulation (Harbuz et al. 1992c). Although one can only speculate that these factors may play a role in the activation of the HPA axis in AA, it is clear that, whatever the mechanism involved, it can largely overcome the feedback inhibition effects of corticosterone.

During the chronic inflammatory stress of AA we also observed differential effects on the expression of pituitary POMC mRNA, which was increased, and pituitary GH and prolactin mRNAs, which were reduced. Changes in GH and prolactin mRNA levels remained unaffected by glucocorticoid status.

Stress leads to increased secretion of GH and prolactin in the rat (Collu et al. 1979; Kant et al. 1987). Concomitant release of glucocorticoids has been shown to modulate the secretion of GH and prolactin during acute stress, although corticosterone effects on these hormones are not as clearly defined as those on ACTH (Noel et al. 1972; Checkley & Arendt, 1984; Delitala et al. 1987). Evidence suggests that the activation of the HPA axis may play a role in the regulation of pituitary GH, since CRH, ACTH and, under certain circumstances, glucocorticoids stimulate GH secretion (Casanueva et al. 1988, 1990; Wiederman et al. 1991).

On the other hand, activation of the immune system in AA leads to inhibition of pituitary GH and prolactin, presumably due to the release of local or circulating inhibitory factors. Intravenous administration of IL-1β or tumour-necrosis factor-α has been shown to result in suppression of GH secretion from the pituitary (Elsasser et al. 1989; Lumpkin & Hartmann, 1989). The effects of IL-1β on prolactin secretion are controversial, some groups finding a reduction or no effect, while others have reported an enhancement (Bernton et al. 1987; Rettori et al. 1987; Beach et al. 1989). Thus, increased production of inflammatory cytokines, such as IL-1β and IL-6, may play an important role in the pathogenesis of the observed changes in the expression of anterior pituitary GH and prolactin mRNAs.

The expression of the pituitary POMC, GH and prolactin mRNAs and their regulation by circulating...
glucocorticoids are seriously compromised in our model of chronic inflammatory AA. The possible role of local or circulating cytokines or other factors in mediating these responses needs further investigation.

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